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Chemo-enzymatically induced copolymerization of phenolics with acrylate compounds

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Abstract Initiation of copolymerization of lignin-like phenolic and acrylic compounds by the phenoloxidase laccase (EC 1.10.3.2) and a peroxide species (*t*-butylhydroperoxide, *t*-BHP) was compared to a Fenton-like system (ferrous ion, *t*-BHP). Initially, the relative activity of laccase towards different phenolic compounds and the optimum pH of some characteristic phenolics were determined. The polymer yield and the average molecular weight (M_w) of chemo-enzymatically produced polymers were dependent both on the type of each phenolic tested and on the phenol/monomer ratio. Furthermore, the success of copolymerization of the phenolics was dependent both on their redox potential and on the type of acrylic monomer applied. The extent of phenol incorporation into the polymer chain was enhanced by the presence of laccase in the reaction mixture and was significantly higher than in polymerization initiated by a Fenton-like reaction.

Introduction

Poly(acrylic) acid and polyacrylamide are highly resistant to bioconversion. Grafting some components into the main polymer backbone may enhance the degradability by linking selected, readily degradable substituents into the polymer chemical structure. In addition to lignin grafting, several attempts have been made in which some naturally occurring polymers of plant or microbial origin, such as starch (Meister 1988), cellulose (Daneault and Kokta 1986), and poly(hydroxybutyric) acid (Dawes 1990) were introduced into a synthetic polymer structure. The naturally occurring fraction of the resulting products have shown appreciable biodegradability. Styrene graft copolymers of lignin were readily degraded by white rot basidiomycetes, while the polystyrene homopolymer was not degradable (Milstein et al. 1992).

Due to their high molecular weight, polymers are not able to pass through the plasma membrane of microbial cells and therefore cannot be degraded inside the cell. One aim of the present study is to incorporate lignin-like phenolic compounds into the polymer backbone to make it susceptible to extra-cellular oxidative enzymes such as laccases and peroxidases. The oxidative degradation of these incorporated phenolics could cause a disintegration of the polymer chain into smaller fragments which could then penetrate the plasma membrane and subsequently be degraded intra-cellularly.

A number of studies have been performed on the graft copolymerization of acrylic monomers onto lignin initiated either by chemical radical starters (Chen et al. 1986; Huang et al. 1992; Meister and Patil 1985; Meister et al. 1984a, b, 1991) or by irradiation (Koshijima and Muraki 1968; Phillips et al. 1972). In all chemical initiator systems, peroxides are applied which form hydroxyl or alkoxyl radicals after homolytic or reductive cleavage of their peroxide bonds. The grafting of either acrylamide (AAm) or acrylic acid (AA) onto water-soluble lignin sulfonates (LS) by the Fenton reaction using water as a solvent has been extensively studied (Chen et al. 1986; Huang et al. 1992). This initiation system led to both high yields and high rates of lignin conversion; however, the resulting polymer mixture had a high homopolymer proportion of 50–60% (Chen et al. 1986).

A chemo-enzymatic process to initiate grafting was recently described, which used a phenoloxidase (laccase) to generate lignin radicals and dioxane peroxides formed by autoxidation of the solvent (Kharazipour et al. 1998; Mai et al. 1999, 2000b). In a subsequent study, the chemo-enzymatic initiation of graft copolymerization with technical LS was compared to a Fenton-like system (Mai et al. 2000a). The term "Fenton-like" was applied because, in this system, the H_2O_2 of the original Fenton reagent was replaced by *t*-butylhydroperoxide (*t*-BHP; Goldstein et al. 1993). This peroxide species did not cause a significant inhibition of laccase as H_2O_2 does.

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The postulated mechanism of chemo-enzymatic grafting describes a contribution of the enzymatically induced lignin radicals either in the formation of alkoxyl (peroxyl) radicals or in a "quenching" of growing acrylamide chains and thus in the formation of covalent bonds between the lignin backbone and the acrylic side chains (Mai et al. 1999).

Chemo-enzymatically induced copolymerization of acrylic and phenolic compounds offers the possibility to produce a new type of engineering material and to achieve a possible alteration of the properties in comparison to conventional polymers. The variation of, e.g. phenol and laccase concentration, presents a means to control the molecular weight of the copolymers under moderate reaction conditions. Moreover, the application of simple lignin-like phenolics provides further insight into the mechanism involved in the chemo-enzymatic grafting of lignin.

In this paper we compare the copolymerization of acrylamide and acrylic acid and phenolics initiated by a Fenton-like reaction to the initiation of a phenoloxidase/peroxide system.

Materials and methods

Enzyme

Laccase was isolated and purified from the culture filtrates of the white rot fungus *Trametes versicolor* (ATCC 11235) as described by Fåhraeus and Reinhammer (1967). The determination of the enzyme activity was performed photometrically, following the oxidation of 2, 2′-azino-bis-(3-ethylthiazoline-6-sulfonate; ABTS) at 420 nm. The enzyme activity was expressed in units defined as 1 U = 1 µmol ABTS oxidized min^{-1} (ε_{420}=36,000 M^{-1} cm^{-1}; Childs and Bardsley 1975). The relative activity of laccase towards different phenolic compounds was determined by an O_2-Clark-electrode (O_2-electrode control box CB-1B; Hansatech Bachhofer, Germany) at a constant temperature of 30 °C and recorded by a two-channel recorder (BD41; Kipp & Zonen, Germany). In the calibration of the Clark-electrode, the O_2 saturation value of the solution was determined in an open measuring chamber while stirring and set at 1,000 mV. The zero value was determined after the removal of O_2 by sodium dithionite.

The measurement was done in 4 ml of potassium-citrate buffer (0.1 M) at the pH values indicated; and 0.15 mmol of the phenolic compound served as substrate. The reaction was started by the addition of 50 µl laccase solution (0.3 U ml^{-1}, final concentration) and recorded for 10 min. All experiments were repeated twice.

Copolymerization of acrylic and phenolic compounds

Copolymers of acrylamide or acrylic acid were synthesized as described in Mai et al. (2000a). Lignin sulfonate was replaced by various phenolics (amounts and redox potentials are given in Table 1).

Molecular weight determination

The molecular weights of the copolymers were analyzed by gel permeation chromatography, as recently described by Mai et al. (2000a).

UV-Vis spectroscopy

UV-Vis spectra were recorded on a DU 640 spectrophotometer (Beckman, Germany).

Elemental analysis

The carbon and nitrogen content of lignin and copolymers were determined in an elemental analyzer (EA 1108; Carlo Erba Instruments). The acrylamide and lignin sulfonate proportion in the copolymerizate was calculated as recently described by Mai et al. (2000a).

Dialysis

Dialysis was performed in Spectrapore 6 (Serva, Sweden) dialysis tubes from regenerated cellulose with a molecular cut-off of 3,500 Da.

Chemicals

All chemicals used in this study were obtained from Merck (Darmstadt, Germany) and were of analytical purity. Sodium lignin sulfonate was supplied by Roth (Germany).

Table 1 Amounts and redox potentials (Chiavari et al. 1988) of phenolic compounds applied in the copolymerization reaction. ~*e* Estimated

Phenolic	Amount (mg)	Redox potential (V)
2, 3-Dihydroxybenzoic acid	120	~+0.60*e*
2, 4-Dihydroxybenzoic acid	120	~+1.0*e*
2, 5-Dihydroxybenzoic acid Na-salt	137	~+0.60*e*
2, 6-Dihydroxybenzoic acid	120	~+1.0*e*
3, 4-Dihydroxybenzoic acid	120	~+0.60*e*
3, 5-Dihydroxybenzoic acid	120	~+1.0*e*
2, 5-Dihydroxybenzene sulfonic acid K-salt	177	~+0.60*e*
Hydrochinon	86	+0.57
Catechol	118	~+0.53*e*
Gallic acid	147	+0.54
Tannin acid	160	–
Guaiacol	97	+0.77
Vanillin	60	+0.92
Caffeic acid	140	+0.53
Ferulic acid	151	+0.82
o-, *m*-, or *p*-Hydroxycinnamic acid	128	+0.85
1, 3-Dihydroxybenzene	86	+0.94
2, 6-Dimethoxyphenol	149	–

Results

Enzyme activity towards phenolic compounds

Prior to the polymerization experiments, the relative activity of laccase towards different phenolics was determined (Fig. 1). The highest activity of laccase was found with 2, 3-dihydroxybenzoic acid; and this value was set as 1. Most of the other phenolics tested which have two hydroxyl groups or a hydroxyl and a methoxyl group, respectively, in the *ortho*- or *para*- position showed an activity of at least 90% of the highest value determined. The only exception was vanillin with 48% of the highest activity. In those phenolics which have hydroxyl groups in the *meta*- position (2, 4-, 2, 6-, and 3, 5–dihydroxybenzoic acid and 1, 3-dihydroxybenzene) no laccase activity was detected.

Some characteristic phenolic compounds were chosen to test the pH optimum of laccase (Fig. 2). Some compounds, such as vanillin, vanillic acid, and sodium lignin sulfonate showed a discrete maximum at pH 4.5, the reported optimum pH of laccase (Ishihara 1983). All other phenolics tested (vanillic alcohol, guaiacol, 2, 5-dihydroxybenzoic acid, 2, 6-dimethoxyphenol, and syringic acid) showed the highest activity at low pH values of 2.5–3 and the activity continuously decreased with increasing pH. A similar dependence of the laccase activity on the substrate is described elsewhere (Fukushima and Kirk 1995; Palmieri et al. 1993). Water-soluble sodium lignin sulfonate showed an optimum pH at 4.5.

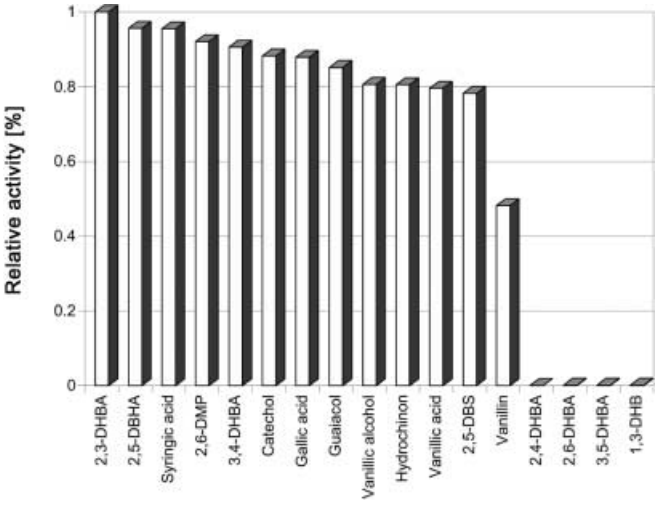

Fig. 1 Relative activity of laccase towards different phenolic substrates (potassium-citrate buffer, 0.1 M, pH 4.5). *DHBA* dihydroxybenzoic acid, *2, 6-DMP* 2, 6-dimethoxyphenol, *2, 5-DBS* 2, 5-dihydroxybenzene sulfonic acid K-salt, *1, 3-DHB* 1, 3-dihydroxybenzol

Yields of copolymerization

Different lignin-like phenolic compounds were tested for their ability to be copolymerized with AAm and AA (Fig. 3). Control experiments which omitted either laccase or *t*-BHP did not produce a significant yield (data not shown). These results correspond to previous findings that both laccase and dioxane peroxides were required to initiate grafting of organosolv lignin (Mai et al. 1999, 2000b), whereas the grafting of lignin sulfonate also proceeded in presence of *t*-BHP alone (Mai et al. 2000a).

In addition, both laccase and phenolics were necessary to induce copolymerization through the generation of alkoxyl or peroxyl radicals from *t*-BHP. A reaction mixture without phenolics did not show any polymerization.

All polymers obtained were water-soluble. The copolymerization of *ortho*- and *para*-substituted dihydroxybenzoic acids (2, 3-, 2, 5-, and 3, 4-DHBA) resulted in a high yield either with AAm and with AA. *Meta*-substituted dihydroxybenzoic acids (2, 4- or 2, 6-DHBA) did not produce copolymerization products with AA, whereas high polymer yields with AAm were achieved by a combination of laccase and the Fenton-like reaction; in the case of 2, 6-DHBA, an enzymatic initiation also led to a significant yield.

The copolymerization of gallic acid, catechol, and dihydroxybenzene sulfonic acid-Na-salt (2, 5-DBS) with AA resulted in a high yield at all initiation systems, whereas no significant yield was obtained in the copoly-

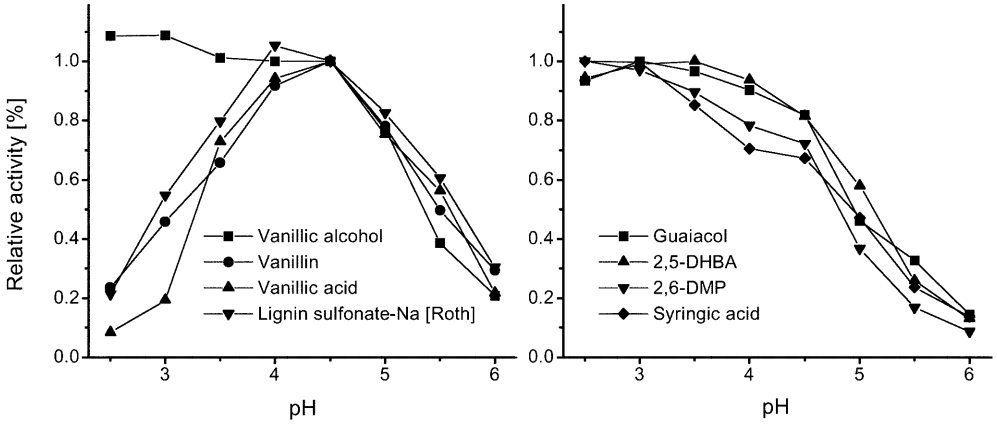

Fig. 2 pH–dependency of laccase with respect to various phenolic substrates (potassium-citrate buffer, 0.1 M). In the left-hand graph, the activity at pH 4.5 was set to 1; in the right-hand graph, the activity at the highest pH was assigned a value of 1. *2, 6-DMP* 2, 6-dimethoxyphenol, *2, 5-DHBA* 2, 5-dihydroxybenzoic acid

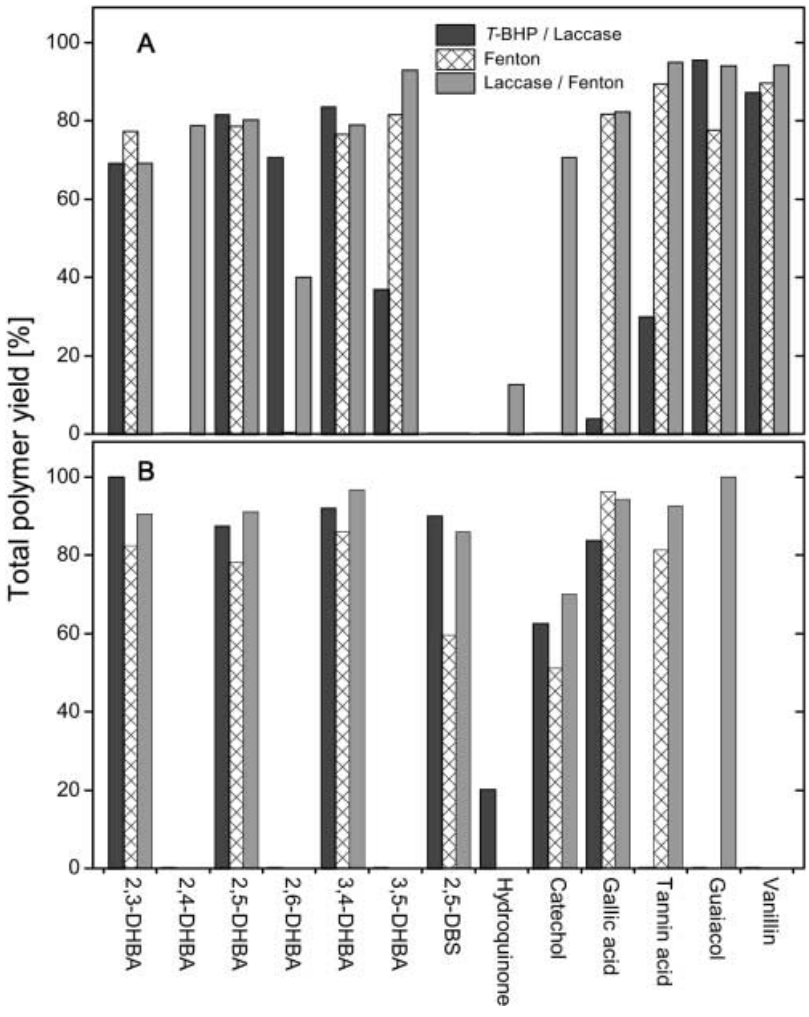

Fig. 3 Yield of copolymerization of acrylamide (**A**) and acrylic acid (**B**) with phenolic compounds produced by different initiator systems. The reaction was run in 8 ml water at pH 4.5 and pH 4.0, respectively, over 48 h; and the initiation system consisted of *t*-butylhydroperoxide (*t*-BHP; 0.15 mmol), laccase (12 units) and $(NH_4)_2Fe(SO_4)_2 \cdot 6H_20$ (1.79 µmol). *2, 5-DBS* 2, 5-Dihydroxybenzene sulfonic acid K–salt

merization of these compounds with AAm. The only exception was the copolymerization of gallic acid and AAm initiated by a Fenton-like reaction.

Some phenolics, such as guaiacol and vanillin, induced a high yield with AAm but not with AA. Also the Fenton-like reaction failed to induce AA copolymerization with these phenolics. Only the combination of the Fenton-like reaction and laccase yielded in the copolymerization of guaiacol and AA, while this combination of initiators did not induce copolymerization of vanillin and AA. A similar phenomenon was observed with 2, 4- and 2, 6- DHBA. No AA copolymers of 2, 4- and 2, 6-DHBA were formed, regardless of the initiation system applied. In AAm copolymerization, laccase, either alone (2, 6-DHBA) or in combination with a Fenton-like reaction (2, 4- and 2, 6-DHBA), was necessary to obtain higher yields; however, the Fenton-like reaction alone did not produce any significant yield.

Other phenolics, such as caffeic acid, ferulic acid, *o*-, *m*-, *p*-hydroxycinnamic acid, 1, 3-dihydroxybenzene (1, 3-DHB), and 2, 6-dimethoxyphenol (2, 6-DMP), only resulted in low or even no yield with both AAm and AA.

Copolymer composition

The composition of AAm copolymers of different phenolic compounds was determined by elemental analysis (Table 2). The extent of phenol incorporation into the polymer backbone was relatively low and ranged over 1–9%, whereas the initial phenol portion in the monomer mixture was about 10%. In almost all cases, it was observed that laccase as part of the initiation system enhanced the extent of incorporated phenols in the copolymerization with AAm. In order to also confirm these results with the AA copolymers, UV-Vis spectra of some products were recorded (Fig. 4). The spectra of AAm copolymers showed a higher absorption in those products which were synthesized either by laccase (and *t*-BHP) alone or by laccase in combination with a Fenton-like reaction; these results correspond to those observed in the copolymerization of AAm (Table 2).

In the AA copolymers, the spectra generally showed a lower absorption in comparison to the AAm copolymers. Here, the initiation by a Fenton-like reaction appears to induce only a small portion of phenol incorporation into the AA backbone. In contrast to the AAm copolymers, the enzymatic initiation of the copolymerization of gallic acid

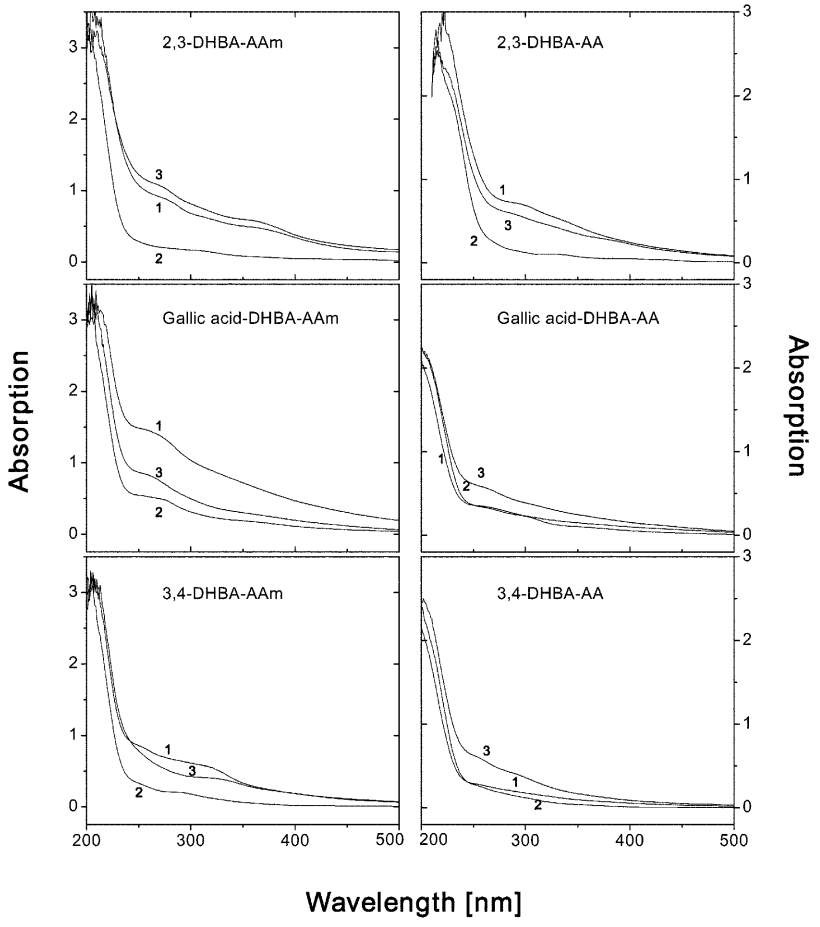

Fig. 4 UV-Vis spectra of polymerization products synthesized by different initiator systems: *1* laccase/*t*-BHP, *2* Fe(II)/*t*-BHP, *3* laccase/Fe(II)/*t*-BHP. *AA* Acrylic acid, *AAm* acryamide

Table 2 Composition of phenol-acrylamide (*AAm*) copolymers initiated by different initiator systems in the presence of *t*-butylhydroperoxide and redox potential of phenolics tested (Chiavari et al. 1988). *DHBA* Dihydroxybenzoic acid

Phenolic	Initiator system	Phenol content (wt%)	AAm content (wt%)
2, 3-DHBA	Laccase	3.3	96.7
2, 3-DHBA	Fe(II)	2.0	98.0
2, 3-DHBA	Fe(II)/lac.	3.9	96.1
2, 4-DHBA	Fe(II)/lac.	1.1	98.9
2, 6-DHBA	Laccase	1.1	98.9
2, 6-DHBA	Fe(II)/lac.	5.4	94.6
2, 5-DHBA	Laccase	2.9	97.2
2, 5-DHBA	Fe(II)	2.8	97.2
2, 5-DHBA	Fe(II)/lac.	8.3	91.7
3, 4-DHBA	Laccase	3.3	96.7
3, 4-DHBA	Fe(II)	1.3	98.8
3, 4-DHBA	Fe(II)/lac.	2.2	97.8
3, 5-DHBA	Fe(II)/lac.	1.9	98.1
Guaiacol	Laccase	5.6	94.5
Guaiacol	Fe(II)	1.4	98.6
Guaiacol	Fe(II)/lac.	5.6	94.4
Tannic acid	Fe(II)/lac.	3.2	96.8
Gallic acid	Fe(II)/lac.	3.6	96.4

and 3, 4-DHBA with AA did not induce a higher phenol incorporation than the Fenton-like reaction. However, such an increase was observed in the copolymerization of 2, 3-DHBA. The combined initiation of both laccase and the Fenton-like reaction generally resulted in a higher extent of incorporation than did the Fenton-like reaction alone.

Average molecular weight

The AAm and AA copolymers showed a strong variation in their average molecular weight ($\bar{M}_w$) depending on the phenol applied as co-monomer (Tables 3 and 4). It was generally observed that the initiation of laccase and *t*-BHP alone yielded a higher ($\bar{M}_w$) with both acrylic monomers than the induction by the Fenton-like reaction or a combination of both initiator systems, respectively. This difference was especially high in the AA copolymers (Table 4).

In contrast to most other phenolics, the copolymerization of guaiacol and AAm induced by the combination of enzymatic and Fenton-like reaction led to a significantly higher ($\bar{M}_w$) (205,200 g mol^{-1}) than the corresponding Fenton-like reaction alone (93,300 g mol^{-1}). Moreover, the level of guaiacol incorporated in the former reaction (5.6%) is much higher than in the latter (1.2%). Apparently, a cross-linking of guaiacol moieties in different AAm chains took place resulting in an increase in the ($\bar{M}_w$).

Table 3 Average molecular weight (M_w) (g mol^{-1}) of phenol-AAm copolymers synthesized by different initiator systems. *t-BHP* *t*-Butylhydroperoxide

Phenolic	Laccase/*t*-BHP	Fenton reagent (*t*-BHP)	Laccase/Fenton reagent (*t*-BHP)
2, 3-DHBA	183,100	112,000	101,000
2, 4-DHBA	–	–	338,400
2, 5-DHBA	85,627	47,500	42,400
2, 6-DHBA	377,500	–	9,100
3, 4-DHBA	237,100	77,400	85,100
3, 5-DHBA	–	69,900	63,400
Tannic acid	–	39,200	173,200
Gallic acid	–	54,900	46,600
Guaiacol	190,000	93,300	205,300
Vanillin	341,700	480,000	362,900

Table 4 Average molecular weight (M_w) (g mol^{-1}) of phenol-AA copolymers synthesized by different initiator systems. *DBS* Dihydoxybenzene sulfonic acid

Phenolic	Laccase/*t*-BHP	Fenton reagent (*t*-BHP)	Laccase/Fenton reagent (*t*-BHP)
2, 3-DHBA	333,900	192,300	145,000
2, 5-DHBA	292,900	105,800	125,600
2, 6-DHBA	–	–	630,000
3, 4-DHBA	400,200	165,200	156,500
Tannic acid	–	235,900	312,800
Gallic acid	393,100	78,300	85,600
Guaiacol	–	–	349,300
2, 5-DBS	290,300	151,000	152,600
Catechol	220,800	77,100	55,600

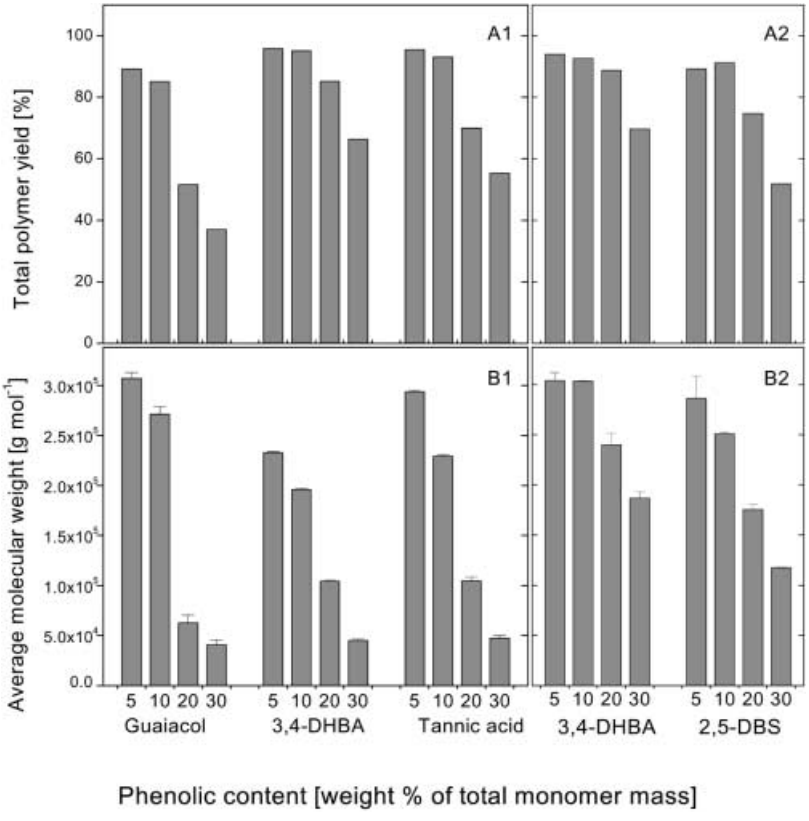

Fig. 5A, B Influence of the phenol portion on polymer yield and the average molecular weight (M_w) of phenol-AAm- and AA-copolymers (**A1** yield of AAm copolymers, **A2** yield of AA copolymers–, **B1** (M_w) of AAm copolymers, **B2** (M_w) of AA copolymers

Ratio of phenol/acrylic monomer

An increasing phenol portion in the initial monomer solution of both AAm and AA resulted in a decrease in both the yield and the (M_w) of the polymer products (Fig. 5). A comparison of the copolymerization of 3, 4-DHBA shows that an increasing phenol portion in the initial monomer mixture causes a higher reduction in the (M_w) of AAm copolymers (Fig. 5B1) compared with the (M_w) of AA copolymers (Fig. 5B2). Since the enzyme ac-

tivity in the AAm mixture appeared to be significantly higher than in the AA mixture, the lower (M_w) can be explained by a higher production of phenoxyl radicals during the AAm polymerization.

Discussion

Enzyme activity towards phenolic compounds

The activity of laccase towards the phenolics tested (Fig. 1) was dependent on their redox potential. Those phenolics with two hydroxyl groups or a hydroxyl and a methoxyl group, respectively, in the *ortho*- or *para*-position showed the highest activities. The activity towards vanillin was significantly lower, since it carries an aldehyde group in addition to the hydroxyl and methoxyl groups. This group exerts a strong electron attracting effect and thus enhances the redox potential of vanillin. However, there was no direct correlation between the redox potential and the activity determined. Thus, laccase showed a lower reactivity with respect to hydroquinone (redox potential of +0.57 V) than with respect to guaiacol (+0.77 V) or vanillin alcohol.

No activity was detected in those phenolics which have hydroxyl groups in the *meta*- position, since these phenolics cannot be oxidized to quinones and they show a relatively high redox potential (Chiavari et al. 1988). However, incubation of these compounds with laccase over several hours led to a coloration of the solution, indicating that a certain degree of oxidation took place.

The pH optimum (Fig. 2) was dependent on the structure and redox potential of the phenolics tested. A comparison of the pH dependency within the guaiacol derivatives shows that an optimum pH at 4.5 appears in those compounds which have a carbonyl (vanillin) or a carboxyl group (vanillic acid). In contrast, vanillic alcohol with a benzyl alcohol group and guaiacol without a further functional group have their highest activity at a low pH. Obviously, the optimum pH of 4.5 appears only if the redox potential of the phenolic compound exceeds a certain level, e.g. if the α-carbon atom possesses an electron-attracting group.

It could be expected that native lignin shows the same pH dependency as vanillic alcohol, because the α-positions of most phenolic groups in lignin are occupied by a hydroxyl group. A testing of the pH-dependency of native lignin was not feasible due to its water-insolubility. Lignin sulfonate, however, showed a pH optimum at 4.5. Obviously, in this type of lignin, most of the α-hydroxyl groups are substituted by sulfonate groups which exert an electron attracting effect similar to that of carbonyl and carboxyl groups.

Yields of copolymerization

As reported previously, the grafting of lignin required a combination of laccase and peroxide species such as dioxane hydroperoxide, *t*-BHP or cumene hydroperoxide (Kharazipour et al. 1998; Mai et al. 1999, 2000a, b). *t*-BHP and cumene hydroperoxide in concentrations of 1.7 mol l^{-1}, which were generally applied in the copolymerization experiments, caused only a minor reduction in enzyme activity (Mai et al. 2000a).

The polymer yields with regard to special phenolic compounds were dependent on the acrylic monomer tested (Fig. 3) and were not directly correlated with the laccase activity towards these phenolics (Fig. 1). *Ortho*- and *para*-diphenols have a low redox potential (between +0.5 V and +0.6 V), while phenolics, which only have a single hydroxyl group (and no methoxyl group) or two hydroxy groups in the *meta*- position, have a redox potential between +0.8 V and +1.10 V (Table 1, Chiavari et al. 1988).

The copolymerization of AA was initiated by phenolic compounds with a low redox potential; and these did not instigate a significant yield with AAm. Thus, the copolymerization of gallic acid (+0.54 V), catechol (+0.53 V) and 2, 5-DBS (+0.60 V, estimated) with AA resulted in a high yield at all initiation systems, whereas no significant yield was obtained in the copolymerization of these compounds with AAm. The only exception was the copolymerization of gallic acid and AAm initiated by a Fenton-like reaction. These results indicate that the ability of phenoxyl radicals to induce radical formation from *t*-BHP is different in aqueous solution of AA from those in AAm solution.

Those phenolics possessing a relatively high redox potential, such as guaiacol (+0.77 V) and vanillin (+0.92 V), induced a high yield with AAm but not with AA. The low yields in the AA copolymerization are probably due to a reduction of the oxidizing potential of laccase in aqueous AA solution. However, the Fenton-like reaction also failed to induce AA copolymerization with these phenolics. A possible explanation for this dependence on the solvent is that in AA solution the standard potential of the iron ion (+0.771 V) is reduced due to chelatization by AA molecules. That is why Fe(III) ions formed in Fenton's reaction cannot be back-reduced by phenolics, whose standard potential is too high. We assume that phenoxyl radicals of these phenols generated by laccase are able to perform this reduction and so maintain the catalytic cycle.

Phenolics, such as 2, 4- and 2, 6-DHBA, which have a high redox potential of at least 1 V (estimated according to Chiavari et al. 1998) could be successfully copolymerized with AAm either with laccase alone (2, 6-DHBA) or in combination with a Fenton-like reaction (2, 4- and 2, 6-DHBA). Apparently, the redox potential of these phenolics is too high to back-reduce Fe(III) ions. However, the corresponding phenoxyl radicals generated by laccase seem to be able to perform this back-reduction and to maintain the catalytic cycle.

Average molecular weight

The higher ($\bar{M}_w$) of copolymers produced in presence of laccase and *t*-BHP alone can be attributed to the lower

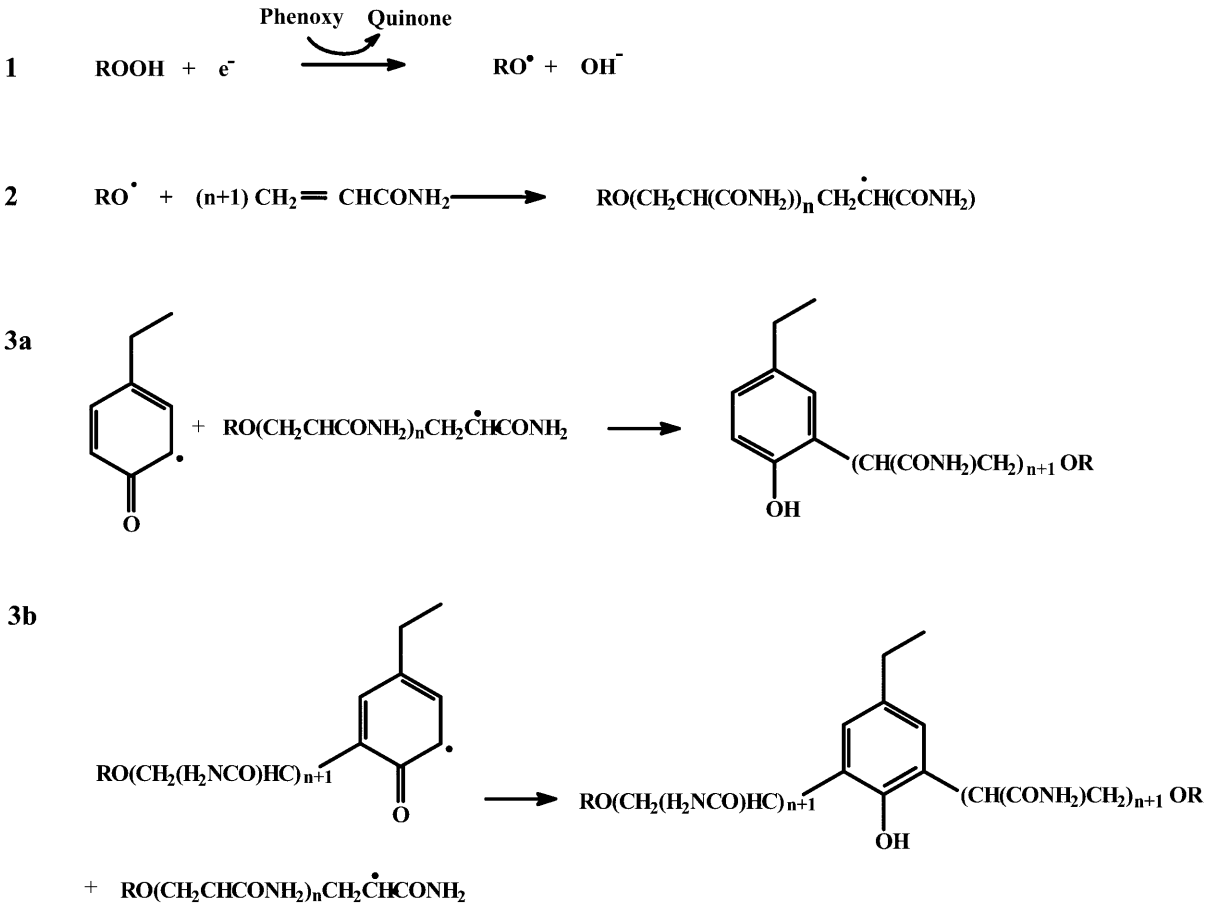

Fig. 6 Proposed mechanism of chemo-enzymatically induced graft copolymerization

number of acrylic chain initiations. In all types of induction systems, the chain initiations are induced by alkoxyl or peroxyl radicals which are formed as a consequence of the decomposition of *t*-BHP. In the Fenton-like reaction alkoxyl radicals are produced by the reaction of *t*-BHP and ferrous ions. Apparently, this reaction proceeds with a much higher velocity than the reduction of *t*-BHP by enzymatically generated phenoxyl radicals (Fig. 6, step 1).

The especially high ($\bar{M}_w$) of the AA copolymers which were enzymatically synthesized can be due to a relatively low enzyme activity in the aqueous AA solution; and this low level of activity resulted in a small number of chain initiations.

If the incorporation of phenolic compounds into the polymer backbone proceeds by a simple coupling of phenoxyl radicals with "living" acrylic chains as recently described (Fig. 6, step 3a, Mai et al. 1999), the extent of phenol incorporation should increase with decreasing ($\bar{M}_w$) However, the data show that the higher portion of incorporated phenols which was achieved in the presence of laccase can not be attributed to a shorter chain length. Although the Fenton-like reaction produced short chain length, the extent of incorporated phenolics was lower than in those reactions in which laccase contributed to the initiation. This indicates that in the Fenton-like reaction, a high number of "living" acrylic chains were terminated by reactions such as disproportionation, H-abstraction, or the coupling of two radical ends of these chains rather than by coupling with phenoxyl radicals. Obviously, through the combination of enzymatic and Fenton-like induction the additional production of phenoxyl radicals by laccase causes an enhanced incorporation of phenolics which is not automatically accompanied with the generation of shorter chain length compared to the "pure" Fenton-like reaction.

Ratio of phenol/acrylic monomer

Phenolic compounds are reported to act as inhibitors of free radical polymerization and are therefore generally applied as stabilizers (radical scavengers) to prevent the unintentional polymerization of liquid monomers (Elias 1981; Falbe and Regitz 1995). That is why the phenolics applied may have an effect on the yield as well as on the average molecular weight ($\bar{M}_w$) of the resulting copolymer.

An increasing phenol portion in the initial monomer solution of both AAm and AA resulted in a decrease in the yield of all phenol copolymers (Fig. 5A), which was much more distinct than that of lignin sulfonate graft copolymerization (Mai et al. 2000a); and this can be as-

sumed to be the result of the low portion of free phenolic sites in lignin. Only about every fourth monomer unit of native lignin bears a free phenolic (hydroxyl) group (Erickson et al. 1973).

In accordance with the proposed mechanism of chemo-enzymatic grafting (Mai et al. 1999, Fig. 6), a higher production of phenoxyl radicals causes, on the one hand, both an increased rate of alkoxyl radical generation and of chain initiations and, on the other hand, a higher rate of chain termination by phenoxyl radicals. Both processes lead to a decrease in (M_w). The data show that the (M_w) of AAm and AA copolymers can be controlled by the concentration of phenolics in the reaction mixture. However, this control proceeds at the expense of polymer yield.

Thus, the copolymerization of 2, 6-DHBA with AAm which was synthesized by a combination of enzymatic and Fenton-like initiation resulted in a very low (M_w) of 9,143 g mol^{-1} and a molecular weight at the peak maximum (M_p) of 1,922 g mol^{-1}. Polyacrylates and polyacrylamides of low molecular weight are of particular interest with regard to industrial application, e.g. in the treatment of industrial effluents, such as drilling muds, co-builders in washing powders, or dispersion agents. A method for the production of low molecular weight polyacrylamides with an M_p of about 1,000 g mol^{-1} was recently described (Bortel et al. 1994). This method required high amounts of H_2O_2 (4–6 wt% of the monomer mixture) and the addition of Cu^{2+}-salt at high temperatures (60–95 °C); these reaction conditions are difficult to realize on an industrial scale. However, chemo-enzymatic copolymerization offers the possibility to produce low molecular weight copolymers under reaction conditions which are easy to control with a simultaneous control of (M_w) and M_p.

In accordance with the proposed mechanism of chemo-enzymatic lignin graft copolymerization (Mai et al. 1999) the incorporation of phenols is achieved through the radical combination of a free electron at the end of a growing "living" acrylic chain with that of a phenoxyl radical (Fig. 6, step 3a). This mechanism was confirmed by the finding that the extent of phenol incorporation into the polymer backbone was enhanced when laccase was part of the initiation system. Both laccase (and *t*-BHP) alone and the combination of laccase with a Fenton-like reaction appear to produce a higher concentration of phenoxyl radicals in the reaction mixture and, as a consequence, a higher portion of phenolics in the resulting polymers. Since the growing acrylic chains are terminated by the phenolics, it can be expected that they are located at the end of the chain. However, if a second growing acrylic chain is "quenched" by a phenolic site which is already located at the end of one chain, the resulting polymer would carry a phenolic site within the chain (Fig. 6, step 3b). In almost all cases, the Fenton reaction produced the lowest molecular weights. Thus, the higher portion of incorporated phenols in presence of laccase cannot be explained by shorter chain length. The data also indicate that phenolic groups in lignin are crucial sites of grafting in the chemo-enzymatic copolymerization of lignin and acrylic compounds.

Although phenolic compounds are known to be radical scavengers and stabilizers, our data show that the copolymerization of AAm and AA with phenolics can be induced either chemo-enzymatically by laccase and a peroxide species (*t*-BHP) or by a Fenton-like reaction. To our knowledge, such kind of copolymerization has not been reported so far. Our data provide a method to produce new types of water soluble polymers with such potential applications as drilling muds, co-builders in washing powders, or dispersion agents. The reaction conditions applied were moderate and made it possible to control the molecular weight of the copolymers by varying phenolic and laccase concentration. However, the main reason for the incorporation of phenolic compounds into the polymer backbone is to make it susceptible to a microbial attack. If the phenols are statistically distributed over the polymer chain, they present sites for the possible attack of extra-cellular oxidative enzymes such as laccases and peroxidases. This oxidative attack can cause the polymer chain to break apart into low molecular weight fractions, which are then able to pass through the membrane of microbial cells where they are degraded intra-cellularly. Initial experiments showed an increased biodegradability of phenol copolymers in comparison to the corresponding acrylic homopolymer (Kharazipour et al. 1998).

Further studies on the biodegradation of phenol-acrylic copolymers as well as on the biodegradation of lignin graft-copolymers are in progress.

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